December 21, 1952

Dear Boris:

Thank you for your note on my review. Of course a reprint will have been reserved for you, and it will be sent as soon as the (very limited) supply is received. I am sure I know what you mean about our points of view-- but converge is hardly the correct term. More accurately, perhaps, the original development of my own perspective could not help but be profoundly influenced by yours. I do hope something can be done to clear the air of a lot of nonsense about autoreproduction and viruses vs. plasmagenes. The first paragraph of this review is quite explicit in disclaiming original thinking, and rightly so. If I were to do this again today, I would hope to be able to do a more complete job. Too many of the issues were left without a finished discussion.

I wish I had an opportunity for a more detailed discussion with you and Harriett about this: it is becoming more certain by the day that there is a "bridge to classical genetics" in our transduction experiments. You might tell Harriett that her guess was right, that FA isalmost certainly phage carrying genetic material from one host to another, but for this same reason we have no good evidence on the chemical constitution of the bacterial fragments. Only one case of "linked" transduction has come up so far. Bruce Stocker started on it this summer, and I have been following it up. Several genes (Fla_1, $_2$;....) are involved in the formation of flagella, and most of them are quite distinct from the factors which determine the H-antigen that appears when a flagellum is produced. However, Fla_1 appears to be associated with H_1. That is, the experiment $Fla_1^+ H_1^i \times Fla_1^- H_1^b$ under conditions of selection for Fla_1^+ gives both H_1^i and H_1^b progeny, in various proportions. A fairly elaborate "backcross" analysis of the H_1^i progeny through several generations gives fairly definite evidence that they result from the coincidental transduction of two factors (H_1^i as well as Fla_1^+). The association is very much more frequent than chance expectation, whence "linked" transduction. Unfortunately, the combination $Fla_1^- H_1^i$ in the above experiment cannot be selected for, so that the proof is, perhaps, incomplete. We are looking for other examples, and meanwhile trying to study the determinism of the b:i ratio. I had thought this to be fairly fixed for the FA of a given $Fla^+ H^-$ stock, but there is apparently some correlation between the delay in appearance and the proportions of b:i in the motile selections. I would guess this to be due to the interactions of different modifiers carried over in fragments of different sizes.

As you may surmise, I am beginning to commit myself to a definite theory of transduction in Salmonella, but I do not take this as more than a guide to further experiments. Phage infection results in a fairly thorough disruption of the bacterial nucleus, most of which is converted into phage "nuclei". Some of the pieces are, however, incompletely digested but are nevertheless incorporated into phage particles. (We have some evidence that the same particles which transduce also carry lysogenizing capacity). These convey the bacterial fragments to a new cell, which often escapes death by being lysogenized. The most ticklish problem is how the fragment is reincorporated, as it is almost certain that its old homologue is replaced. One suggestion is that the fragment synapses with its homologous segment in the host nucleus, and is incorporated into an intact "chromosome" only at subsequent replications. I say chromosome fragment rather than DNA macromolecule; be it as you wish. However, on purely theoretical (but still sound) grounds, I can see no possibility of the control of bacterial heredity by an unorganized menagerie of determinants, one for each trait, and each independent of the others. Perhaps it is time to attempt transduction experiments in higher organisms with preparations of isolated chromosomes!

Zinder, as you may know, is now at Rockefeller, working with Schneider. I trust he will be imbued there with a sense of the unique significance of DNA in heredity.

Another interesting line of work is the genetic basis of flagellar phase variation in Salmonella. You can look up the natural historical details in Topley and Wilson's textbook. It turns out that only one phase is transducible at one time, whence two loci, H_1 and H_2 are assumed. The question is how it is determined whether H_1 or H_2 will be expressed. I had expected, perhaps, to find a situation like Paramecium where the cytoplasmic state is decisive, and the underlying genotype not altered. In Salmonella, however, only the phase that is phenotypically expressed is detectable in the FA made from it, although the alternative phase is latent in these cells. That is, $H_1^a\ H_2^x$ —x $H_1^b\ H_2^y$ gives only $H_1^a\ H_2^y$, and not $H_1^b\ H_2^x$ or $H_1^a\ H_2^x$ (The phenotypically expressed phase is underlined). For technical reasons, I have not been able, so far, to make a satisfactory study of the role of the phase of the recipient cells in this determination; it appears not to be decisive. The kind of hypothesis that comes out of this confusing story is that the H_1 and H_2 may occur in active or inactive states, themselves (and not merely limited in their final effect, as in Paramecium), for their states stay with the factors in transduction. One could conceive of a cycle from nucleus to cytoplasm back to nucleus to account for the stability of the existing state, but this may be straining an already burdened speculation. This does pose some obvious embryological possibilities, namely persistent states of nuclear factors as elements in differentiation, but entirely distinct from ordinary mutation. The usefulness of this hypothesis for Salmonella is to suggest experiments to control the local states; this has not yet been done.

Throughout the above, as I omitted to mention, a —x b is shorthand for "FA from a is applied to b". A secondary consequence of these experiments is the construction of innumerable "species" or serotypes of the Salmonella group, some previously named, others not.

To contrast with all of the above, E. coli (K-12 and about 50 others) is still "sexual". Tom Nelson is here doing some physiological and kinetic studies with Hfr strains; if we're lucky, we may have some microscopic facts before toomlong, but these might not be so decisive on the question of sexual mechanism as might be hopefully imagined.

To Harriett and yourself, best wishes for the season and the new year.

A theory of the underlying mechanism of transduction in Salmonella is beginning to emerge from the experimental facts, but its present status is not so secure as to be more than a guide for further studies. First, the FA is almost certainly carried by phage particles, as shown not only by attempts at physical separation, but by the coincidence in the production of FA and phage (either by induction of lysogenic bacteria, or lytic growth of phage) and by similarities in the the superficial properties in reactions with antisera and with the binding sites of various bacteria. In addition, there is a distinct correlation between transduction and lysogenization, but the latter process is still very poorly understood. The phage is regarded as a passive vehicle for the genetic material of the bacterium, for the range of traits that a given phage preparation can transduce is limited by the genotype of the bacteria on which the phage has just been grown, and its previous hosts leave no impression after a single cycle of growth on a new host. The association of transduction with phage activity can be disrupted by treatment with ultra-violet light at very high doses which leaves most of the transducing activity intact while removing the plaque-forming capacity of the phage. It is doubtful that there has been a physical separation of FA from phage, but this must still be verified. It seems more likely, rather, that the ability of the phage, qua parasite, to multiply in the bacterium has been impaired without destroying the mechanics of inoculation of the contents of the phage particle into the bacterium. It is not surprising that the FA "fragment" should have a much small effective cross-section than the intact bacterium or the virus. Our information on the intervening steps of transduction is very limited. We know that we can, in the end, recover a small fraction of bacteria (ca. 10^{-5} at most) to which a new trait has been transduced, and there is fairly substantial evidence from serological work that the old homolgous is no longer present. We can only speculate on the details at the present time. With only a single exception [redacted] mentioned [redacted] the transductions of different traits are entirely independent.

My notions about transduction are strongly conditioned by some a priori notions of genetic organization. Although the experiments seem to show that the bacterial genotype can be shattered without destroying the function of the isolated parts, I cannot believe that an organism as complex as a bacterium can survive by virtue of an unorganized menagerie of determinants, one for each heritable function. There is therefore the ticklish problem of reconciling the disorganized FA with the organized bacterial genotype, of finding a mechanism precise enough to allow for the replacement of homologous determinants. This problem was not acute when transformation experiments applied to single or very few characteristics— one can apologize for a few varieties of plasmagenes diffused throughout the cell— but it is becoming increasingly clear that transductions can encompass the entire genotype, piece by piece. There is known one mechanism that may have the necessary precision, namely the synapsis of homologous segments of chromosomes of higher forms at meiosis, and it seems almost necessary to invoke it here. One can thus postulate that "undigested" chromosome fragments are transported by phage particles to new host bacteria, that in these bacteria a fragment may synapse with its homologue, and that it may be incorporated (or be used as a model for) into an intact chromosome at a later replication. We have some evidence that many transductions (of a motility factor) may be abortive, i.e., the new genetic effect is lost after a few cell divisions; it must be kept in mind that we usually see only the successes, and the low efficiency of transduction leaves a good deal of room for the failures. We are still uncertain of the chemistry or the genetic scope of the fragments— a single instance of "linked" transduction, as mentioned tends against the notion that we are dealing with <u>the isolated genes</u>, whatever that may mean.

Another line of work that appears to be promising is a study of flagellar phase variation in Salmonella-- if you wish, you can look up the natural historical details in Topley and Wilson's textbook. The main point is that each serotype shows a pair of flagellar antigens, only one of which is expressed at any one time, and between which there is a mutation-like oscillation at a rate that may be anywhere from 10^{-2} to perhaps 10^{-10} per division. The phase variation is precisely reversible, i.e., one almost invariably gets back the initial phase, so that the serotypic formulas usually present the two alternatives as, e.g. (S. paratyphi B) b:1,2 meaning a reversible variation from b to 1,2 back to b and so forth. Other types may be, e.g., i:1,2 (S. typhimurium) or b:enx (S. abony), so that the character of the second phase does not appear at first glance to be inherent in the first. [I might add that as a medical student, the numerous recombinations of antigens in Salmonella seemed to me a compelling clue of genetic recombination in bacteria, and that the hope of studying the genetics of phase variation was a principal motivation of taking up Salmonella after E. coli].

On the surface, one might well expect to find a situation here as in Paramecium, where the potentialities for alternative phases would all be laid down in the genotype, which does not vary, but whose immediate expression is controlled by extranuclear factors, the cytoplasmic state. This is not contradicted by the first finding, that the phases are transduced separately so that one gets from an experiment such as i:b2 —x b:enx either i:enx or b:1,2 but never i:1,2. This justifies symbolizing two "loci": e.g., H_1^1 H_2^{12}, of which H_1 is already mentioned as linked to Hla_1, but it is not linked to H_2. A paradox only arises when one considers the activities of FA prepared from either the i or 12 phase alone. One would have expected them to be equivalent, but in fact, i:1,2 —x b:enx gives only i:enx (where the expressed phase is underlined) and so on. Unfortunately, I do not yet have suitable material to be entirely sure of the role of the phase of the recipient cells, but this does not seem to be decisive. The experiments have also been done in reiprocal order, namely b:enx —x i:1,2 with comparable results. This would seem to indicate that H_2^{12} is not represented in the FA from i:1,2 cells, although it must certainly be present in some form, as it reappears when i:1,2 is replaced by b, as in a transduction experiment.

These data must appear confusing, but they are consistent with one interpretation (inter alia) which may be more interesting. Unlike Paramecium, it would appear that the state of a locus remains with it after it has been transported to another cell. That is to say, there is a local state of activation or suppression, which tends to persist in heredity, though not indefinitely. One can imagine a steady state cycle through the cytoplasm and back to the nucleus, but this is somewhat ad hoc at present. It may be rather difficult to prove this notion in Salmonella, as we are already leaning heavily on our tentative conclusions about transduction. However, it may afford another model for differentiation (if any more are needed) which is a compromise between differential mutation and cytoplasmic segregation, both of which are untenable in their extreme forms. I shall be surprised if this is an entirely new suggestion.

Norton Zinder, as you may know, is at Rockefeller now working with Schneider, but not entirely isolated from Hotchkiss. I trust he will be well imbued there with the unique significance of DNA in heredity. Tom Nelson is with us now, doing some kinetic and physiological studies with Hfr strains in E. coli recombination. K-12 and some 50 other E. coli strains are still "sexual", although Larry Morse and Esther are working on a unique transduction in K-12, whereby a Gal_4^+ marker can be transduced by lambda. This is the only trait that seems to work--the main reason it had not been picked up before. The sexual and transduction mechanisms are still quite distinct: the former requires the "F+" factor, but not lambda, and the converse. If we are lucky we may have some microscopic images of K-12 recombination, but these may not be so decisive as might be imagined.

To Harriett and yourself, best wishes for the season and the new year.